



**Armed Forces College of
Medicine
AFCM**



Applied Clinical Chemistry of Cardiovascular Diseases

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INTENDED LEARNING OBJECTIVES (ILO)



By the end of this lab the student will be able to:

Interpret the biochemical bases of different clinical disorders related to the cardiovascular system

Case scenario



A *51-year-old* man comes to ER complaining of severe chest pain radiating to his left arm. He *smokes* around 20 cigarettes per day and eats a typical Western diet.



Case scenario



Physical examination reveals that his blood pressure is 150/100 mmHg and pulse rate 75 per minute regular. His chest is clear on auscultation. ECG and ECHO were performed. A blood sample was drawn for analysis of serum cardiac biomarkers.

Define cardiac biomarkers.

Are intracellular proteins (mostly enzymes) specific to the cardiac muscle and released into blood after cell damage. They are used for diagnosis and follow up of MI.

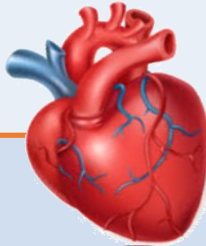
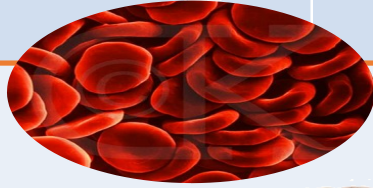
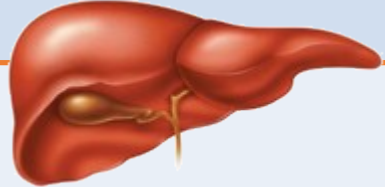
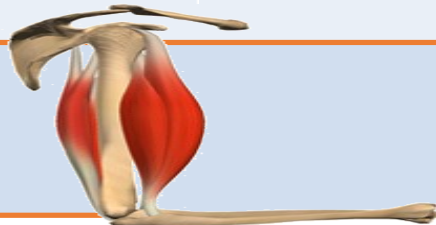
Define isoenzymes.

- *Are enzymes that catalyze the same reaction and act on the same Substrate.**
- *Different forms of the same Enzyme.**
- *synthesized in different tissues.**
- *separated from each other by ELECTROPHORESIS**

LDH isoenzymes



LDH isoenzymes are present in blood in the form of tetramers

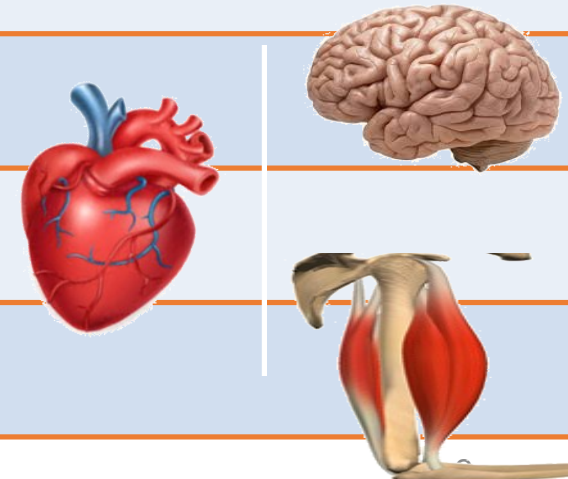
LDH isoenzyme	Tetramer	Specific for
LDH1	HHHH  H ₄ (LDH ₁)	HEART 
LDH2	HHHM  H ₃ M (LDH ₂)	RBCs 
LDH3	HHMM  H ₂ M ₂ (LDH ₃)	BRAIN 
LDH4	HMMM  HM ₃ (LDH ₄)	LIVER 
LDH5	MMMM  M ₄ (LDH ₅)	MUSCLE 

CK isoenzymes



➤ **Three** isoforms are present in the form of dimers

CK isoenzyme	Dimer	Specific for
CK1	BB	BRAIN
CK2	MB	HEART
CK3	MM	MUSCLE



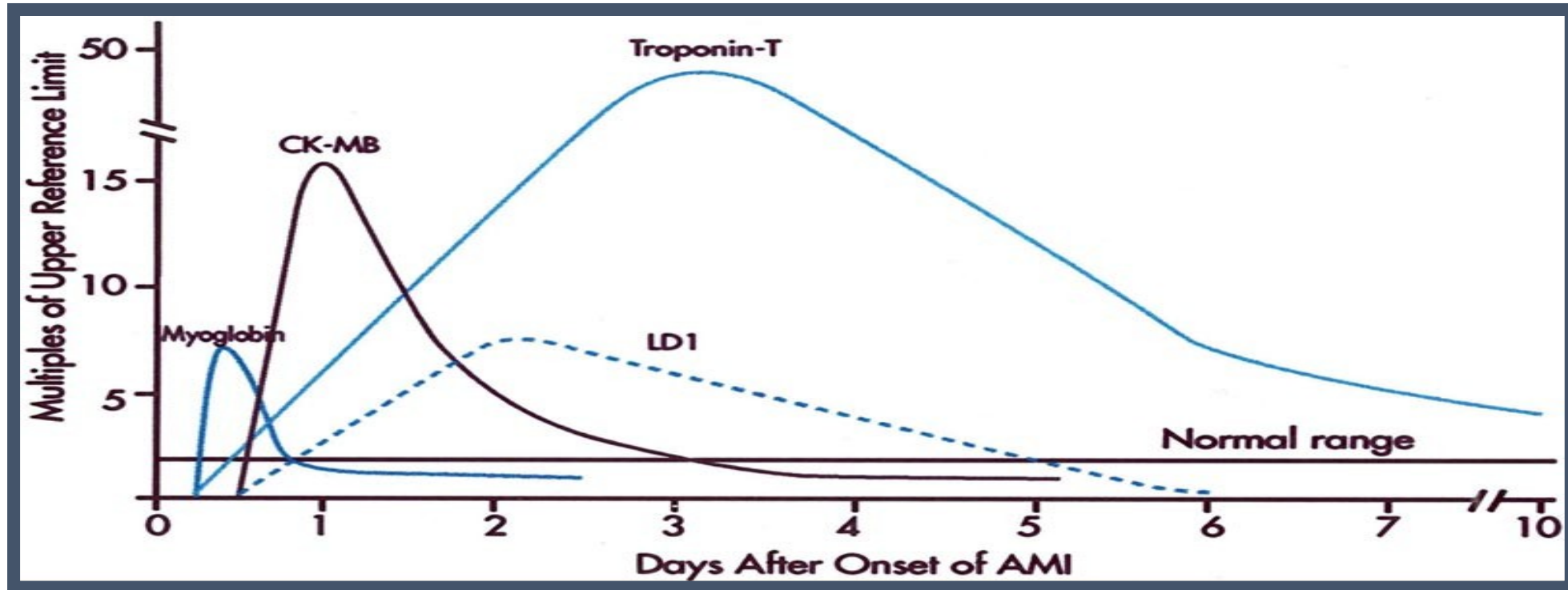
What is the time sequence of cardiac enzyme changes with MI?

Cardiac marker	Rises	Peaks	Returns to normal
Troponin	3-8 hours	1-2 days	3-10 days
CK2	4-8 hours	24 hours	2-3 days
LDH	8-12 hours	3-6 days	8-14 days

What is the time sequence of cardiac enzyme changes with MI?

Cardiac marker	Rises	Peaks	Returns to normal
Troponin			
CK2			
LDH			

Serum Cardiac Marker



0- 4 hrs MI: Myoglobin is released.
4- 48 hrs: CPK, CK-MB, cTnI, cTnT, AST.
> 48 hrs: LDH, cTnT.

Troponin & CK2 are used for early diagnosis of MI while TnT & LDH are used for follow up.

Explain the role of enzymes in treatment of MI?

- ✓ Streptokinase is an enzyme used as a therapeutic agent. It is prepared from streptococcus bacteria.
- ✓ Used in treatment of MI through ~~preteolytic~~ cleavage of plasminogen ~~→~~ converting it to plasmin turning on the fibrinolytic system lysis of blood clots which cause MI.

Discuss the covalent modification of pyruvate dehydrogenase enzyme

Covalent Modification of (PDH)

- PDH active in **dephosphorylated** form and inactive in **phosphorylated** form
- Phosphorylation is catalyzed by PDH Kinase (**cAMP independent**)

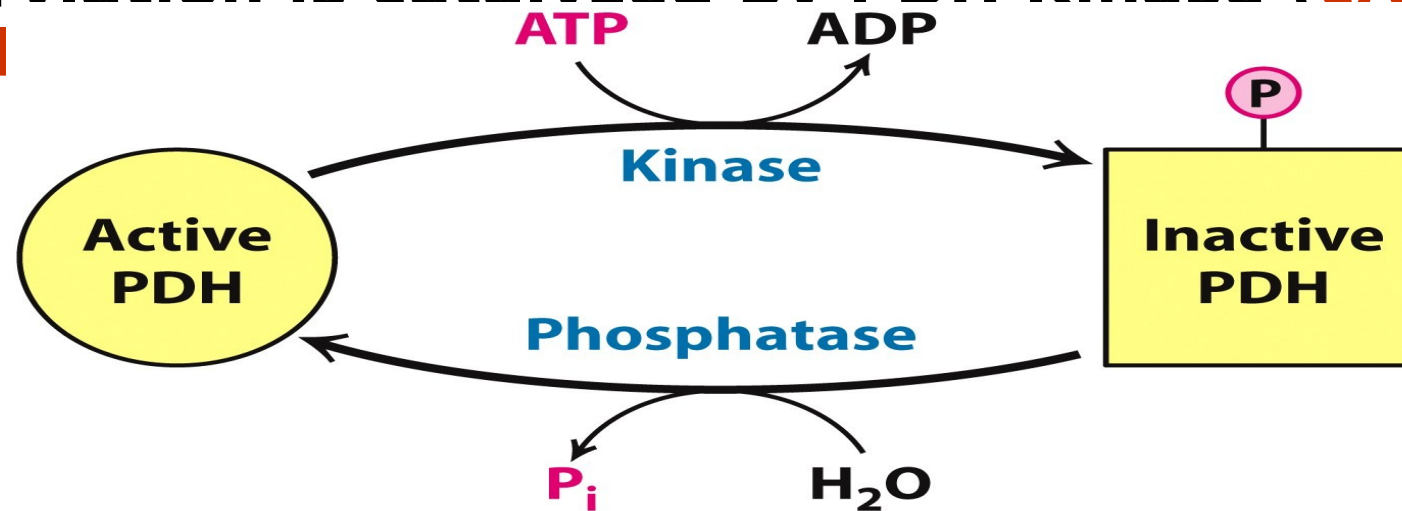
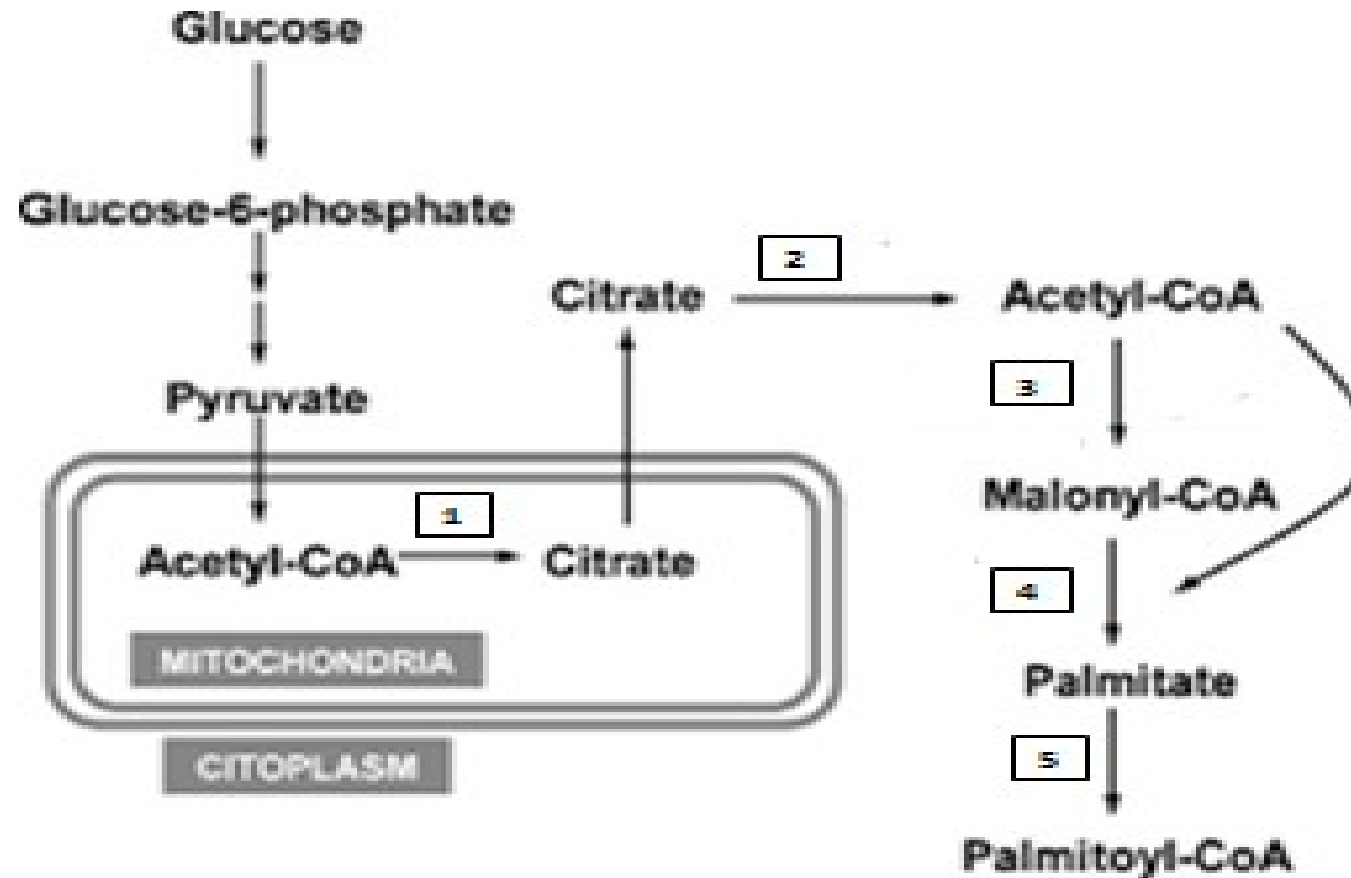


Figure 17.17
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

Mention the missing enzymes as regarding steps of fatty acid synthesis and activation:



1- Citrate Synthase, 2- ATP Citrate Lyase, 3- Acetyl CoA Carboxylase, 4-Fatty acid synthase, 5- Thiokinase

De novo synthesis of fatty acids occurs in:

1. Cytosol
2. Mitochondria
3. Microsomes
4. Nucleus
5. Ribosomes

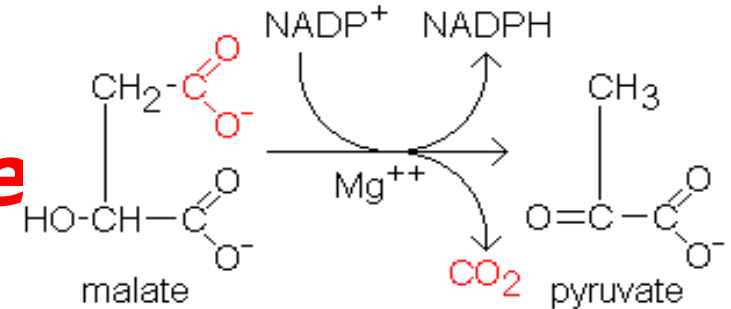
Which of the following is required as a reductant in fatty acid synthesis?

1. NADH
2. NADPH
3. FADH₂
4. FMN H₂
5. Vitamin C

Which metabolic pathway(s) or reaction(s) supply NADPH for FA synthesis?

1-HMP Pathway *
(2 NADPH /one glucose).

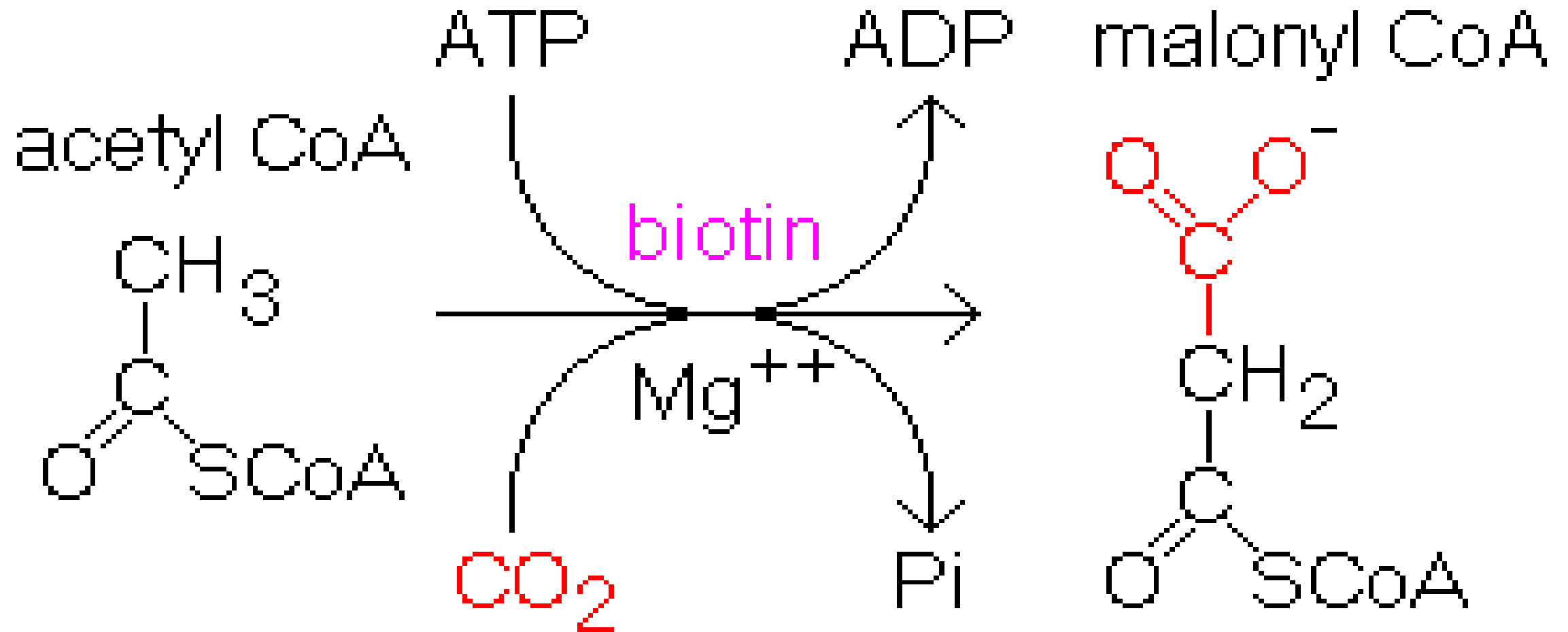
2-Cytosolic Malic enzyme



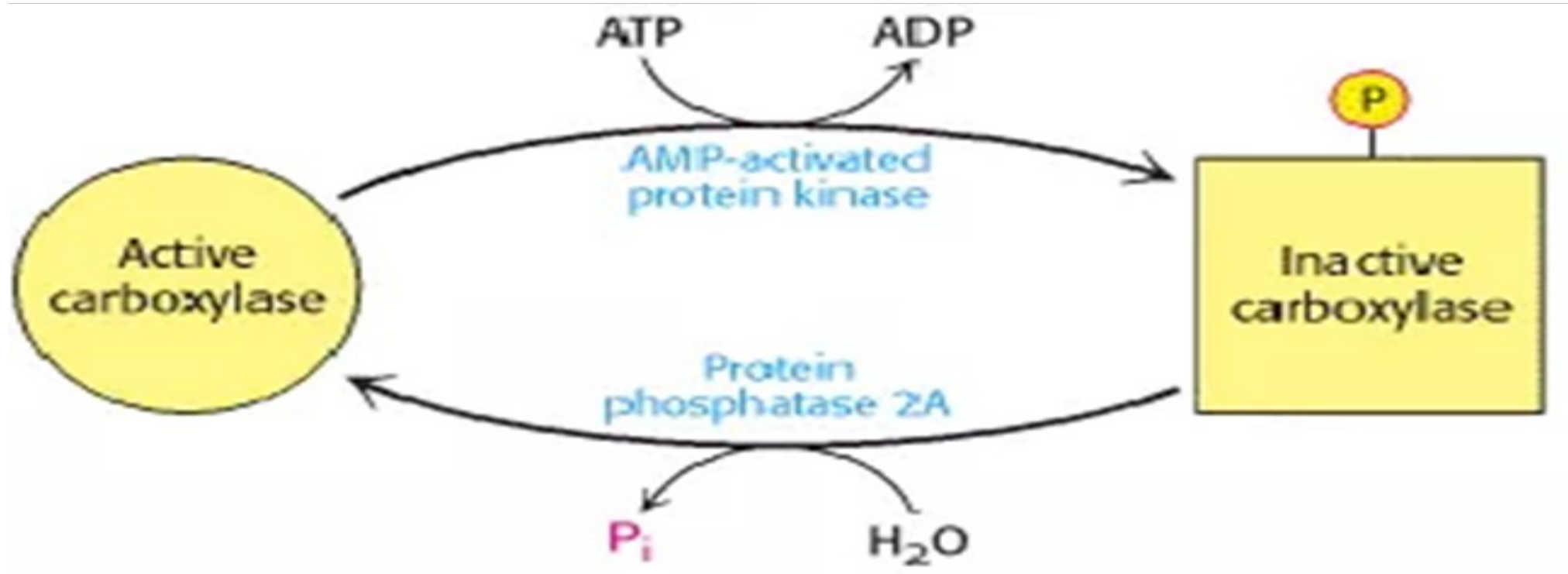
**3-Cytosolic isocitrate
dehydrogenase(minor)
keto glutarate**

Isocitrate α

Q. Illustrate the reaction catalysed by **Acetyl CoA Carboxylase and its covalent regulation.**



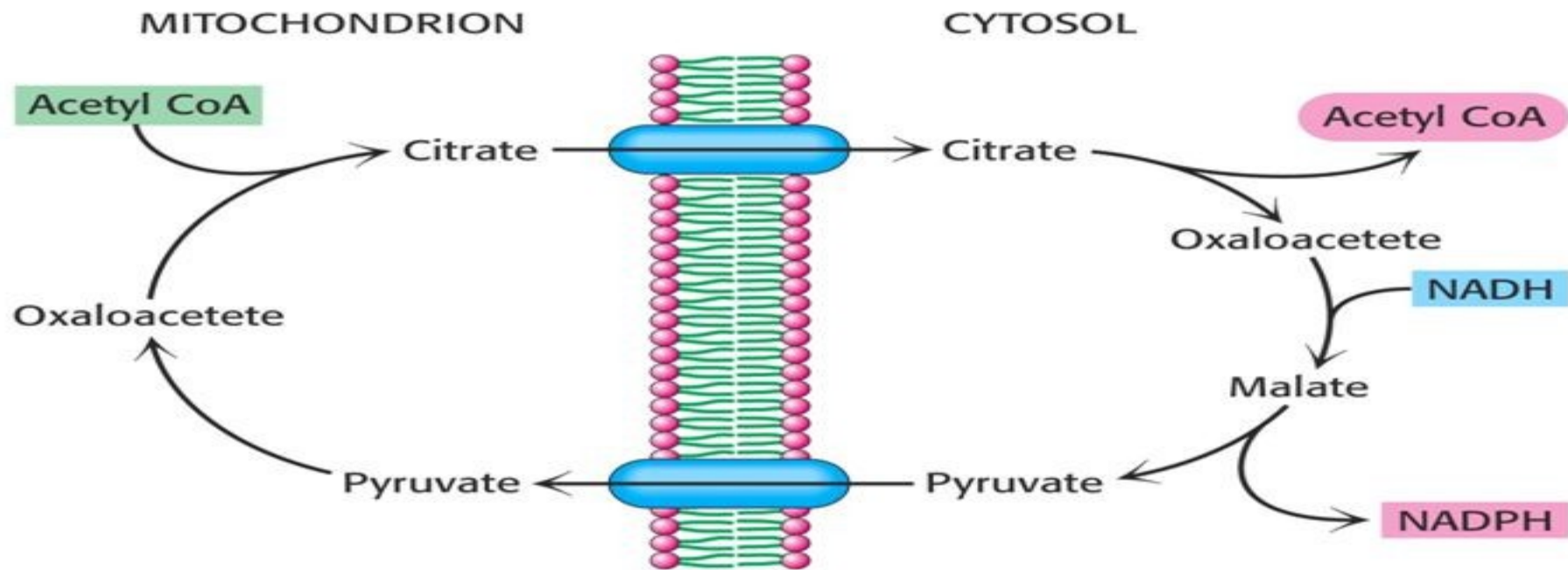
Glucagon, Epinephrine



Insulin

Illustrate: Citrate Shuttle

Mention its importance



For transport of Acetyl CoA from mitochondria to cytoplasm for Cytosolic De Novo Fatty Acid

Match

1. A source of NADPH
2. The rate limiting step in FA synthesis
3. a multienzyme complex

- a) Acetyl CoA Carboxylase
- b) Fatty acid synthase
- c) Malic enzyme

1. c, 2. a, 3. b

A tissue with the most numerous fatty acid elongation capabilities:

1. RBCs
2. liver
3. Cornea
4. Muscles
5. Brain

Case scenario

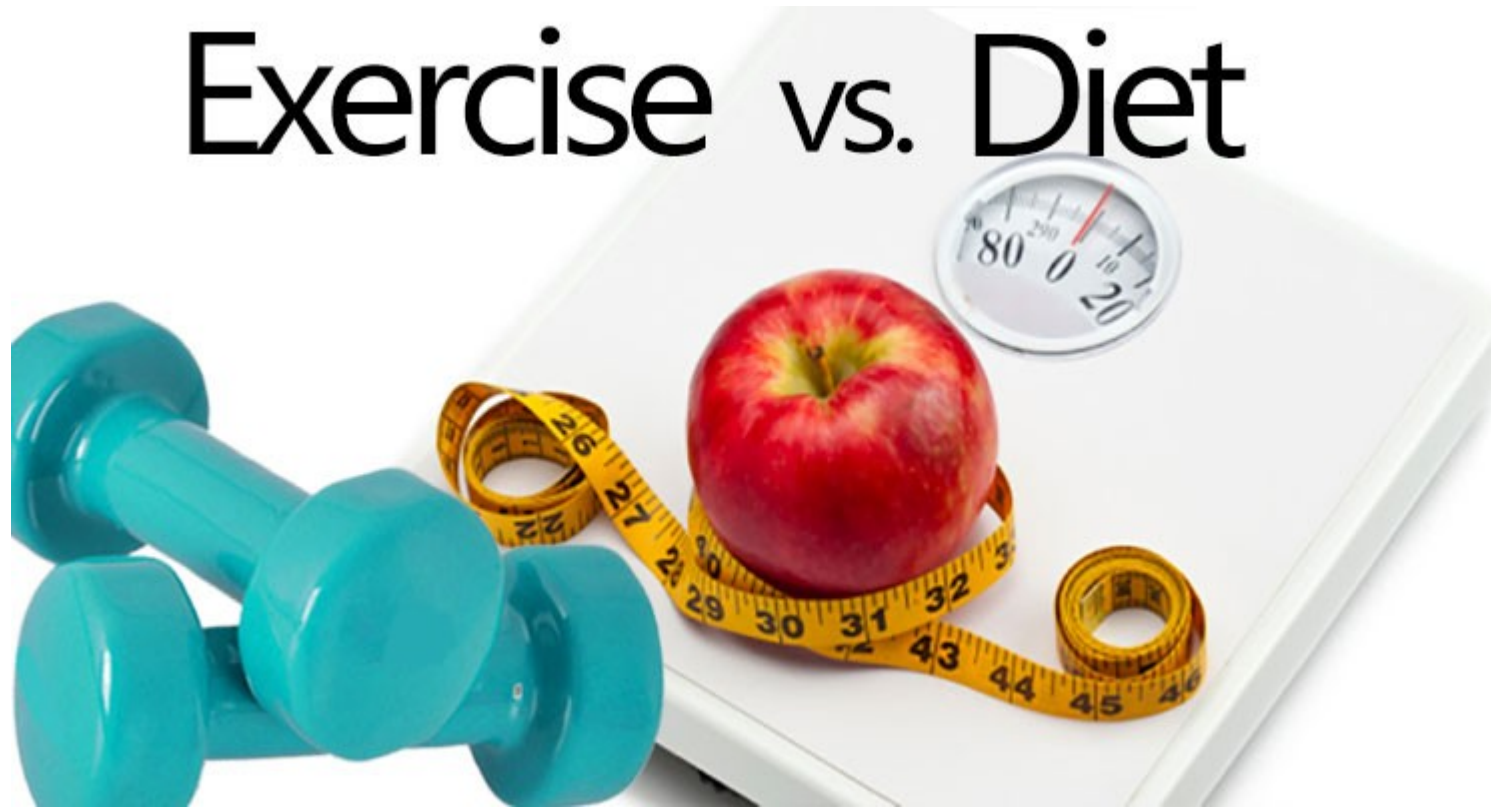


- Sanaa is a 45 year old housewife. She lives a **sedentary type** of life and she **loves to eat**. She hardly does any **exercise**. She has been **gaining a lot of weight** lately, specially in her abdomen, so went to a nutrition doctor.
- She reported that she ate **minimal fatty food**, however she eats a **lot of carbohydrates**. The doctor



- Sanaa followed the instructions and was able to lose 10 kilograms of her extra weight in 5 months.

Exercise vs. Diet



What is the type of lipid stored in this patient's abdomen?

- A. Cholesterol
- ☒ B. Triacylglycerol
- C. Phospholipids
- D. Sphingomyelin
- E. Fatty acids

TAG synthesis involves all of the following enzymes EXCEPT:

- A. Glycerol 3-Phosphate dehydrogenase
- B. Thiokinase
- C. Hormone sensitive lipase**
- D. Glycerol-3P-Acyltransferase
- E. Glycerol kinase

Although the patient reported eating minimal fatty food, yet she gained a lot of fat in her abdomen.

What is your explanation?

**Increased
CHO in
diet → ↑
Insulin**

**↑ GLUT-4 → ↑ glucose
transport → ↑ DHAP → ↑
Glycerol-3-P**

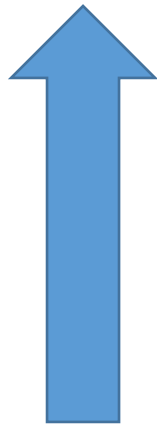
Substrate
Availability (short
term)

Covalent
modifications
(short term)

Hormonal
(long term)

Substrate availability (its effect on ACC)

- Increased CHO in diet → ↑ Insulin



Glycolysis & PDH → Acetyl CoA

HMP pathway → NADPH

ACC, FAS expression



<https://metro.co.uk/2018/03/06/acknowledge-emotional-side-eating-well-never-combat-obesity-7365313/>

Covalent

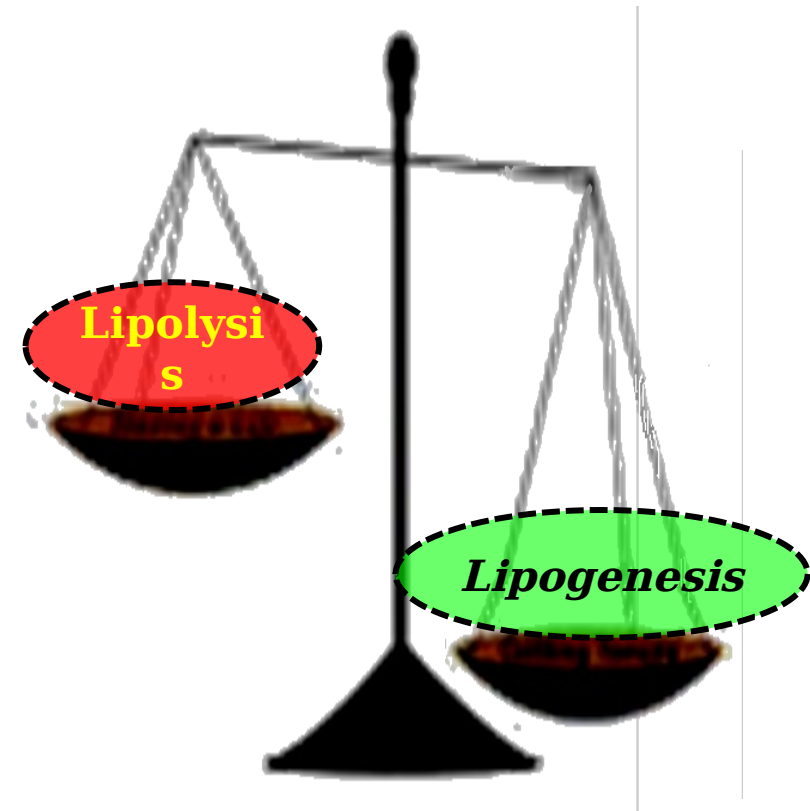
Insulin

↓
↑ cAMP phosphodiesterase,
↑ lipase phosphatase

↓
Dephosphorylation

↓
Active ACC(acetyl COA
carboxylase), , inactive HSL

↓
(↑ TAG synthesis, ↓
Degradation)



**How did dieting and exercise
help Sanaa lose weight?**

Dieting,

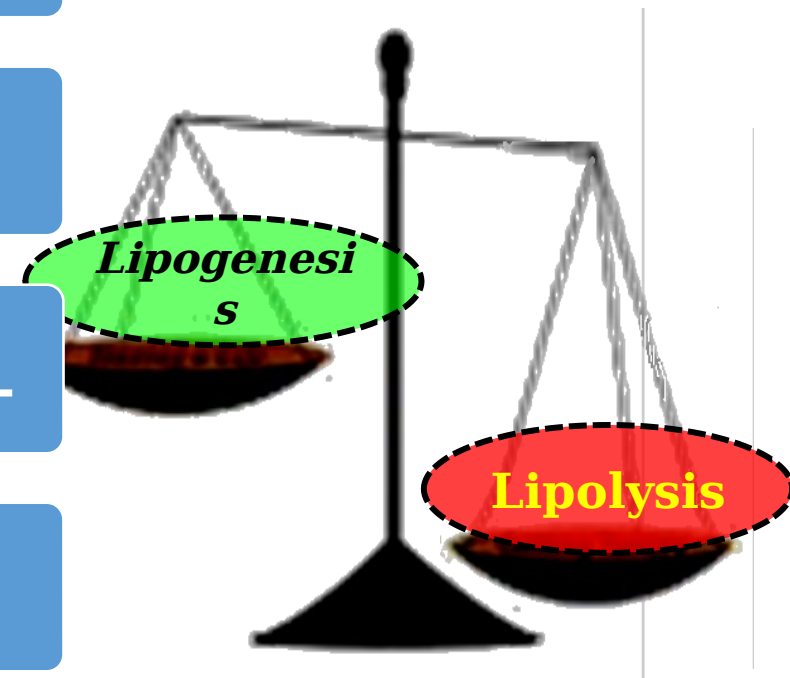
Glucagon, Epinephrin

↑ cAMP

phosphorylation

inactive ACC , Active HSL

↓ TAG synthesis,
↑ Degradation



Which of the following enzymes is present **ONLY** in liver?

- A. Glycerol 3-Phosphate dehydrogenase
- B. Thiokinase
- C. Glycerol-3P-Acyltransferase
- ☒ D. Glycerol kinase
- E. Pyruvate dehydrogenase

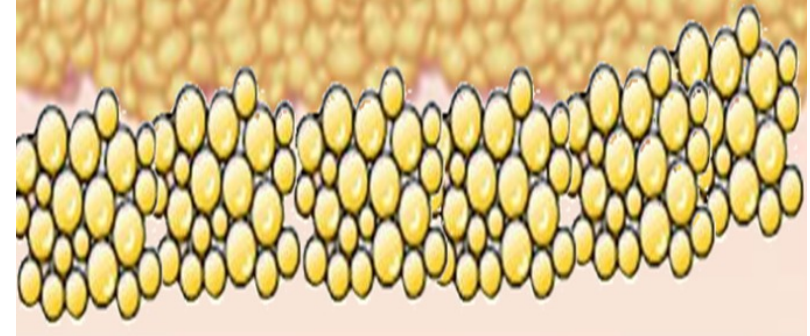
The primary site of TAG synthesis:

1. RBCs
2. liver
3. Cornea
4. Muscles
5. Joints

Explain: After synthesis, TAG have different fates in liver & adipose tissue

1- Adipose tissue

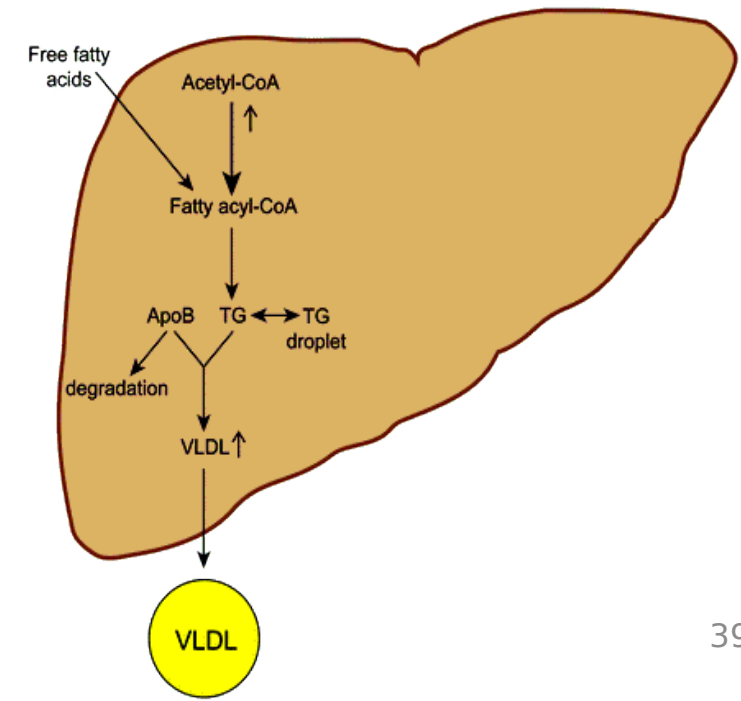
Stored in an anhydrous form ready for mobilization when the body requires it for fuel.



2- Liver

Little TAG is stored in liver.

Most of TAG is exported outside in the form of lipoproteins VLDL (Very Low Density Lipoproteins).



Match

1. Thyroid Hormones
 2. Insulin
 3. Glucagon
 4. Epinephrine
 5. Caffeine & Green Tea
-
- a) ↑ activity of Hormone-sensitive lipase
 - b) ↓ activity of Hormone-sensitive lipase

1, 3, 4, 5 with
A
2 with B

Compare and contrast between fatty acid synthesis and degradation (β oxidation)

	SYNTHESIS	DEGRADATION
Greatest flux through pathway	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Primarily cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymers	NADPH (reduction)	NAD ⁺ , FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β -oxidation
Activator	Citrate	
Inhibitor	Long-chain fatty acyl CoA (inhibits <i>acetyl CoA carboxylase</i>)	Malonyl CoA (inhibits <i>carnitine palmitoyltransferase-I</i>)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction, dehydration, reduction	Dehydrogenation, hydration, dehydrogenation, thiolysis

Case scenario



A seven-year-old schoolgirl who, starting from three years of age, began presenting frequent episodes of **sweating, vomiting, fatigue**, generalized **muscle weakness**, and low glucose levels. These episodes became more recurrent, with shorter intervals between their presentation. The girl also presented growth and language delay.



Laboratory tests showed: Fasting blood glucose: 61 mg/dL (N 100 mg/dL), Levels of **free carnitine** in the plasma were 4.3



Sources of carnitine

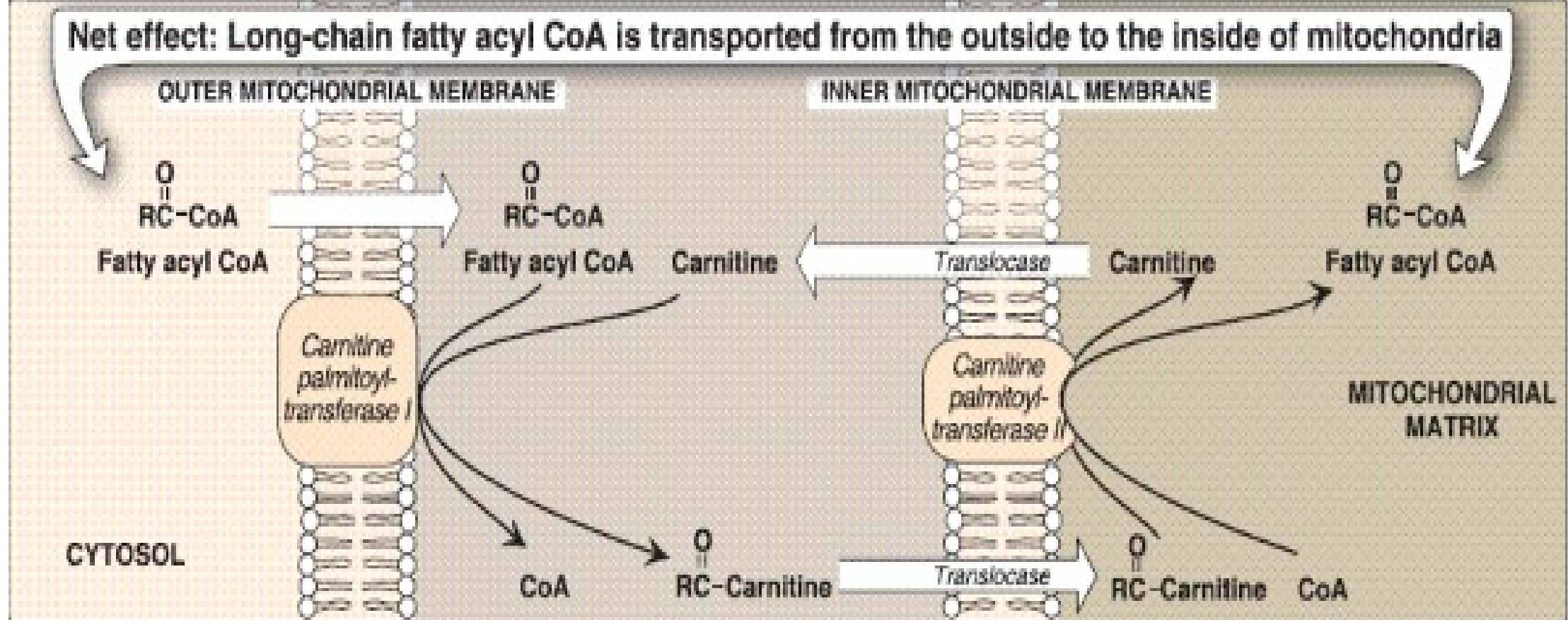
1-diet (meat products).



2- Endogenous synthesized carnitine (lysine and methionine) by liver &kidney but not in skeletal or heart muscles.

LCFA transport (Carnitine Shuttle)

- Carnitine shuttle allow **LCFA** to enter the matrix (as inner mitochondrial membranes are impermeable to CoA).**
 - FAs <12C (e.g milk) passes without the aid of the shuttle.**
- They are activated to their CoA derivatives inside the matrix by matrix enzymes to be ready for oxidation.**



LCFA transport (Carnitine Shuttle):

- 1- Acyl group transferred from CoA to carnitine by ***carnitine palmitoyltransferase I (CPT-I) or CAT-I for carnitine acyltransferase I.***
- 2-acylcarnitine enters the matrix in exchange for free carnitine by ***carnitine-acylcarnitine translocase.***
- 3-***(CPT-II, or CAT-II)*** catalyzes the transfer of the acyl group from carnitine to CoA in the matrix; regenerating free carnitine.

Carnitine deficiencies results in a decreased ability of tissues to use LCFA as a metabolic

Primary carnitine deficiency:
= Congenital deficiencies in any of CPT system

CPT-I deficiency
(liver)

CPT-II deficiency
(cardiac & skeletal ms)

Inability to use LCFA for fuel impairs Gluconogenesis. This leads to: severe hypoglycemia, coma, & death

Cardiomyopathy and muscle weakness with myoglobinemia following prolonged Exercise.

**2ry
carnitine
deficiency**

**Malnutrition patients
(vegetarian)**

**Liver disease Patients
(decreased synthesis of carnitine).**

**Hemodialysis patients
(removes carnitine from the blood)**

**Increased requirement for carnitine
(pregnancy, severe infections, burns, or trauma)**

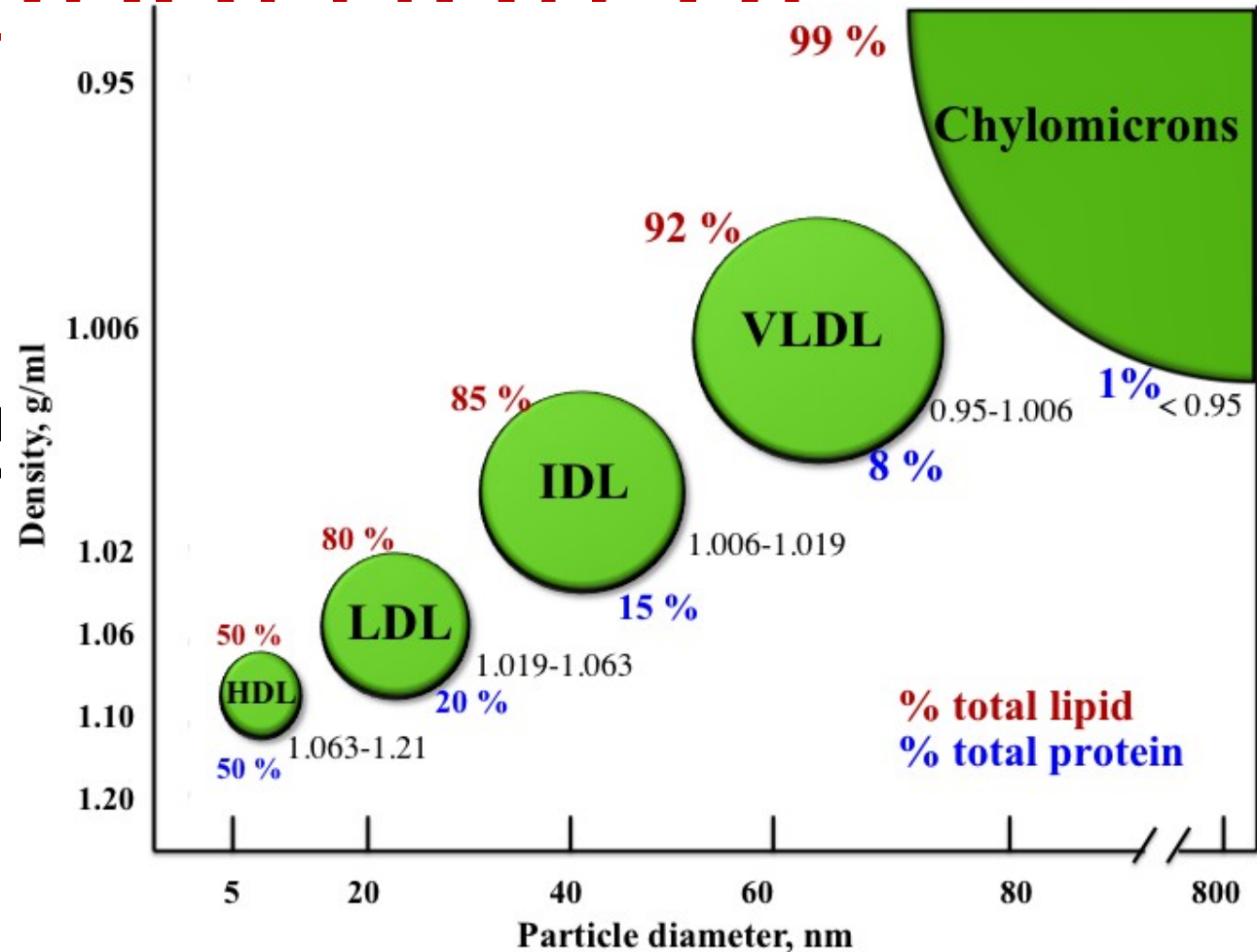
Treatment includes

1. **avoidance of prolonged fasts.**
2. **diet high in carbohydrate and low in LCFA, but supplemented with medium-chain fatty acid.**
3. **in cases of carnitine deficiency, carnitine.**



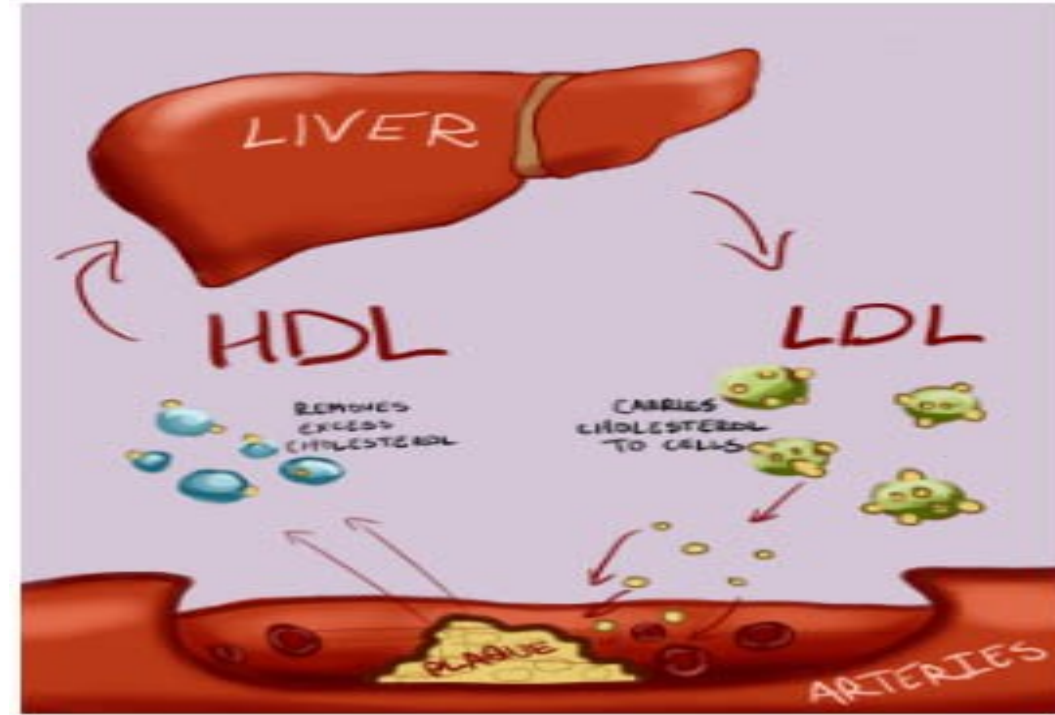
➤ Proteins make the greatest proportion of

- a. VLDL.
- b. Chylomicrons.
- c. IDL.
- d. HDL.**
- e. LDL.



➤ Which type of lipoproteins is most closely associated with atherogenesis?

- .a. Reduced VLDL
- .b. Reduced IDL
- .c. Elevated HDL
- .d. Elevated LDL**
- e. Reduced LDL



<https://freeformfitness.ca/good-vs-bad-cholesterol-is-there-really-a-difference/>

➤ Endogenously synthesized hepatic triacylglycerols are

a. Degraded in the enterocyte before direct transport to the liver.

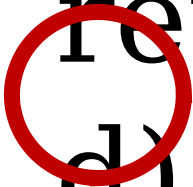
b. Transported to adipose tissue via VLDL.

c. Transported from the enterocyte via chylomicron.

d. Carried to the adipocyte on HDL.

e. Transported to the adipose tissue carried on albumin

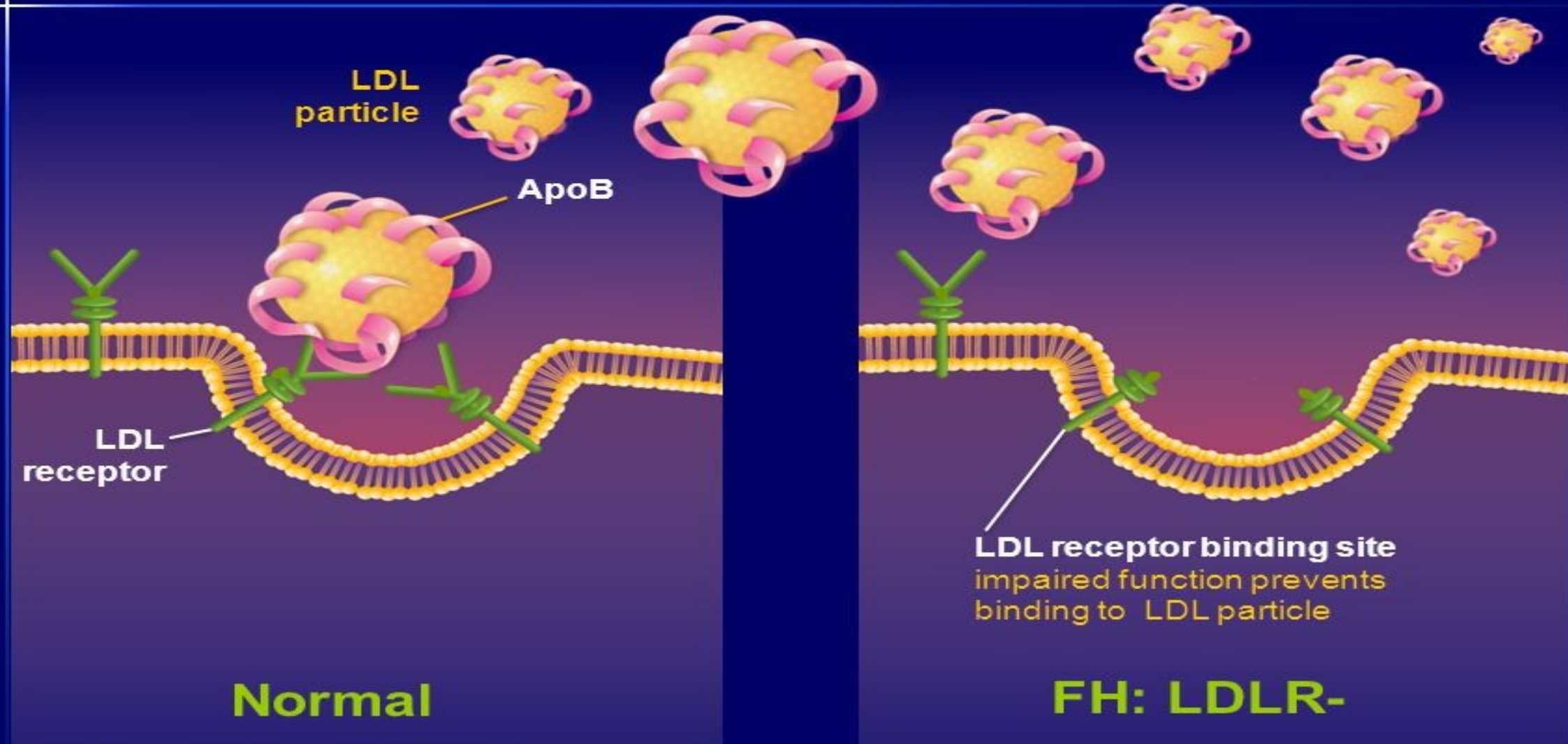
➤ The synthesis of HMG CoA can occur:

- a) Only in the mitochondria.
- b) Only in the cytosol.
- c) Only in the endoplasmic reticulum.
-  d) In cytosol and mitochondria.
- e) In lysosomes.

➤ Which of the following is responsible for familial hypercholesterolemia?

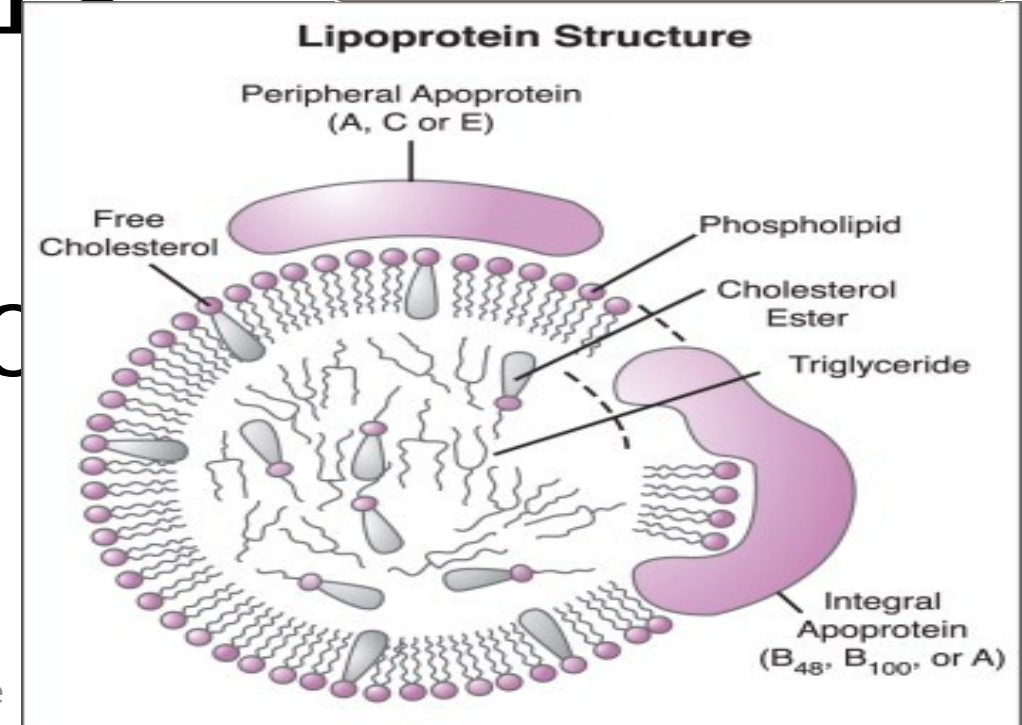
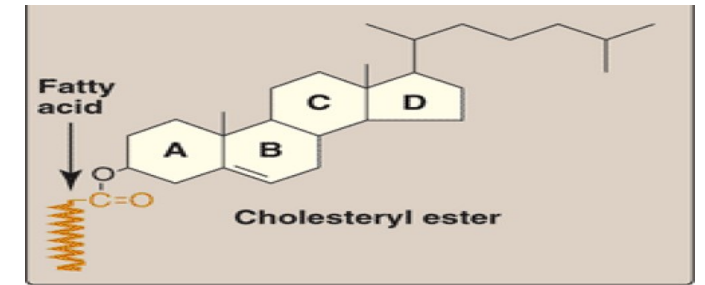
- .a. Over-intake of fatty meals
- .b. Excessive intake of carbohydrate
- .c. Deficiency of lipoprotein lipase
- .d. Deficiency of LDL receptors
- ☒ .e. Lack of intestinal cholesterol esterase

LDL Receptor (LDLR) Mutations Alter LDL Receptors, Preventing Attachment to Particle



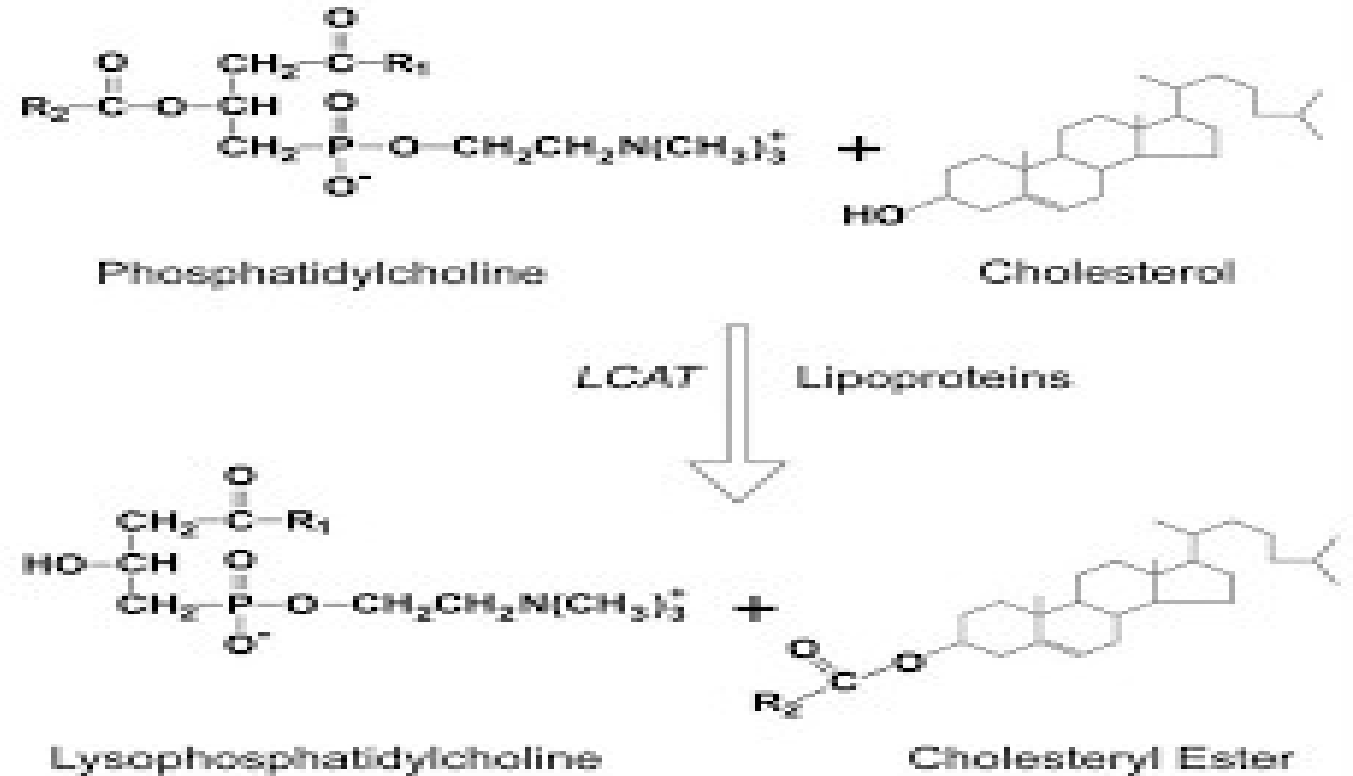
➤ Which of the following may be present in the core of a plasma lipoprotein particle?

- .a. Cholesterol
- .b. Cholesterol esters**
- .c. Apolipoproteins
- .d. Phosphatidyl ch
- .e. Enzymes



➤ The enzyme lecithin-cholesterol acyl transferase is important for cholesterol

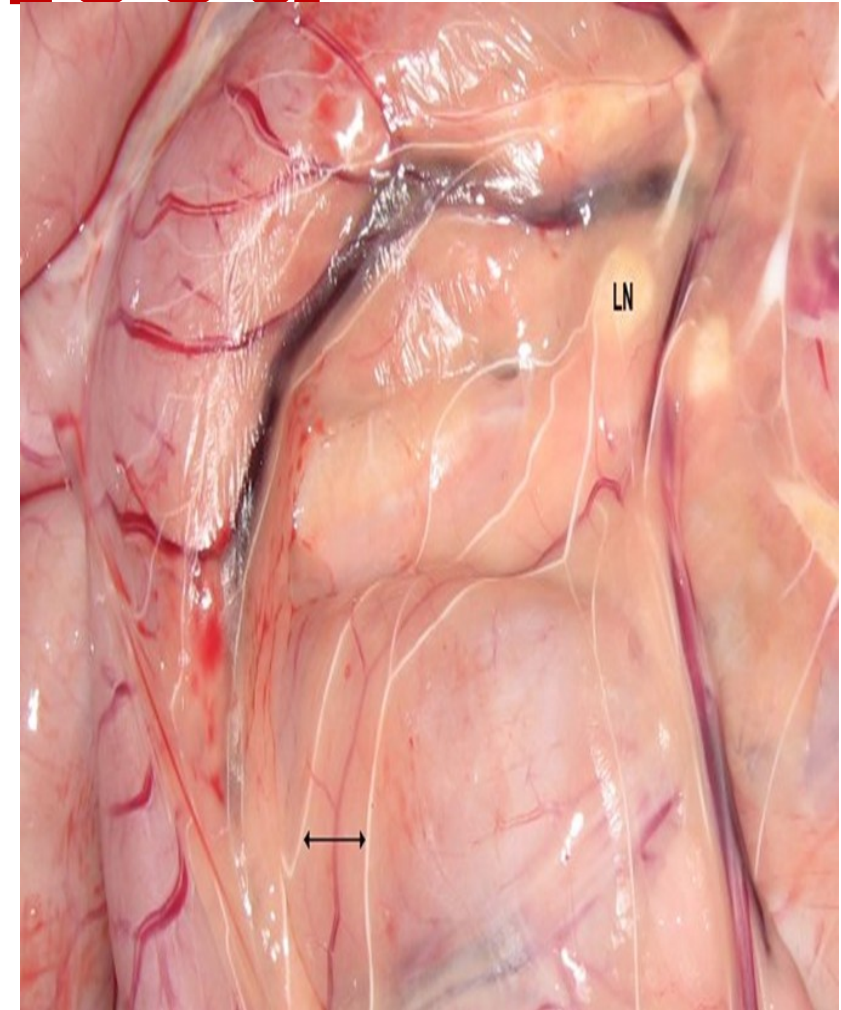
- .a. digestion
- .b. absorption
- .c. oxidation
- .d. esterification**
- .e. storage



<http://www.meditecno.pt/Upload/Product/Archive/STA-616-lcat-elisa-kit.pdf>

➤ Which of the following plasma components is responsible for lipemia that follows food intake?

- ☒ a. Chylomicrons
- b. LDL
- c. HDL
- d. VLDL
- e. Lipoprotein lipase



➤ Which of the following may be undergone by cholesterol?

- .a. Oxidation for energy production
- .b. De novo synthesis of fatty acids
- .c. Formation of glucose by gluconeogenesis
- .d. Esterification to share in membrane structure
- ☒ e. Incorporation in VLDL particles produced by liver cells

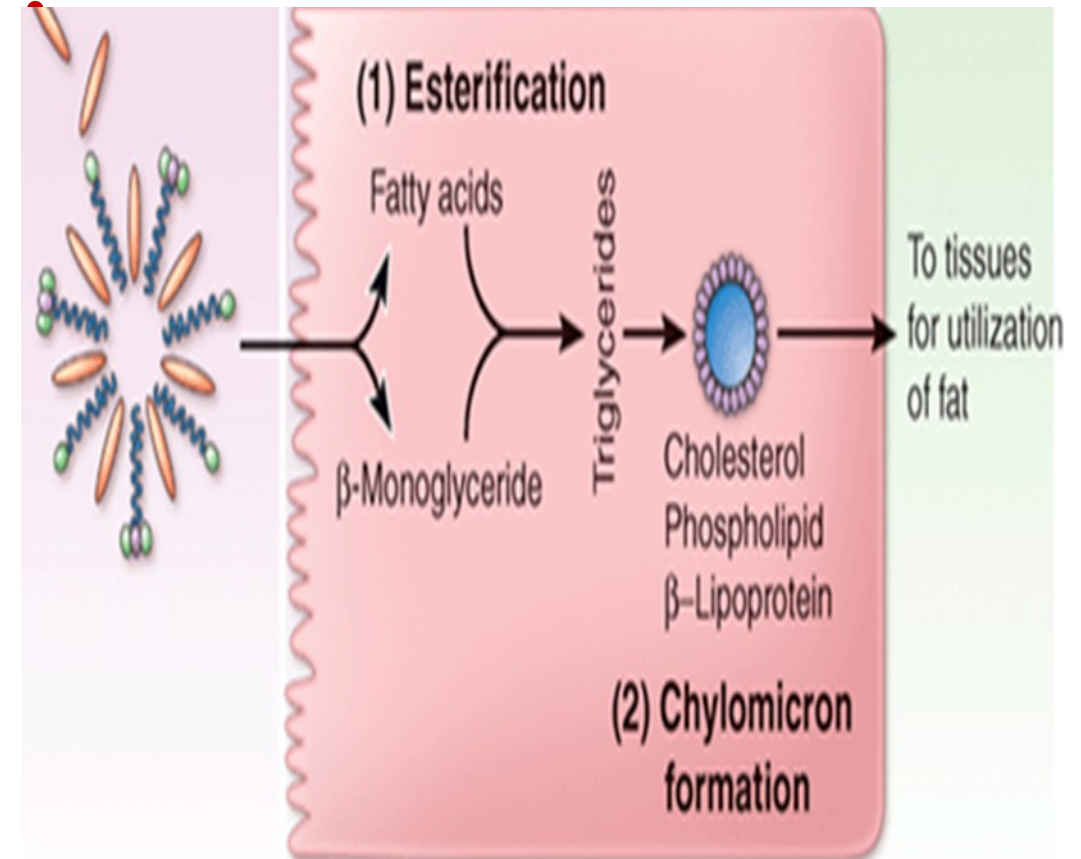
➤ Apoprotein A-1 (Apo A-I) is an activator of

- .a. Lecithin: cholesterol acyltransferase
- .b. Acyl-CoA: cholesterol acyltransferase
- .c. Lipoprotein lipase
- .d. Cholesterol ester transfer protein
- .e. Phospholipid transfer protein

Apo A-I → ↑ LCAT
Apo B → Receptors for LDL
Apo C → ↑ LPL
Apo D → ↑ CETP
Apo E → Receptors for Chylomicrons

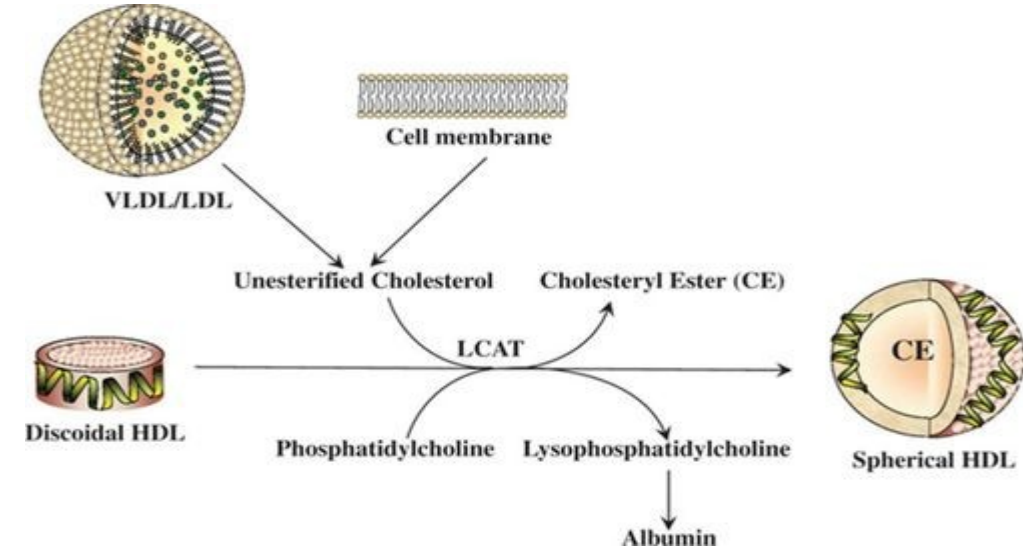
➤ Triacylglycerols resynthesized in the intestinal mucosal cells (enterocytes) are transported by:

- .a. Albumin
- .b. LDL
- .c. VLDL
- .d. Chylomicrons**
- .e. HDL



➤ Atherogenesis is NOT promoted by:

- a. Diabetes.
- b. Cigarette smoking.
- c. Macrophage action.
- d. Elevated lecithin-cholesterol acyltransferase (LCAT) activity.**
- e. Presence of reactive oxygen species.



Case scenario



- A 52 year old man came to the outpatient clinic complaining from **persistent fatigue** and **headache**. On examination his blood pressure was found to be **170/110 mmHg**(average range: 120/80 mmHg). His hands and eyelid had yellowish **xanthomas**).



New Five Year Program



- When the patient was asked about his **eating habits**, he reported eating a lot of French fries from fast food restaurants, biscuits and burgers. **(TRANS-FAT)**
- He also reported suffering once from severe **chest pain**, but he just rested and did not seek medical consultation.
- The doctor asked the patient to go to the lab the following day after 12 hours fasting to check his blood lipid profile.
- Fasting blood **cholesterol** level turned out to be **500 mg/dl** (Normal < 200 mg/dl)

What is your explanation of the patient's condition?

Severe hypercholesterolemia

```
graph TD; A[Severe hypercholesterolemia] --> B[atherosclerosis]; B --> C[Hypertension]; C --> D[headache]; C --> E[fatigue];
```

atherosclerosis

Hypertension

headache

fatigue

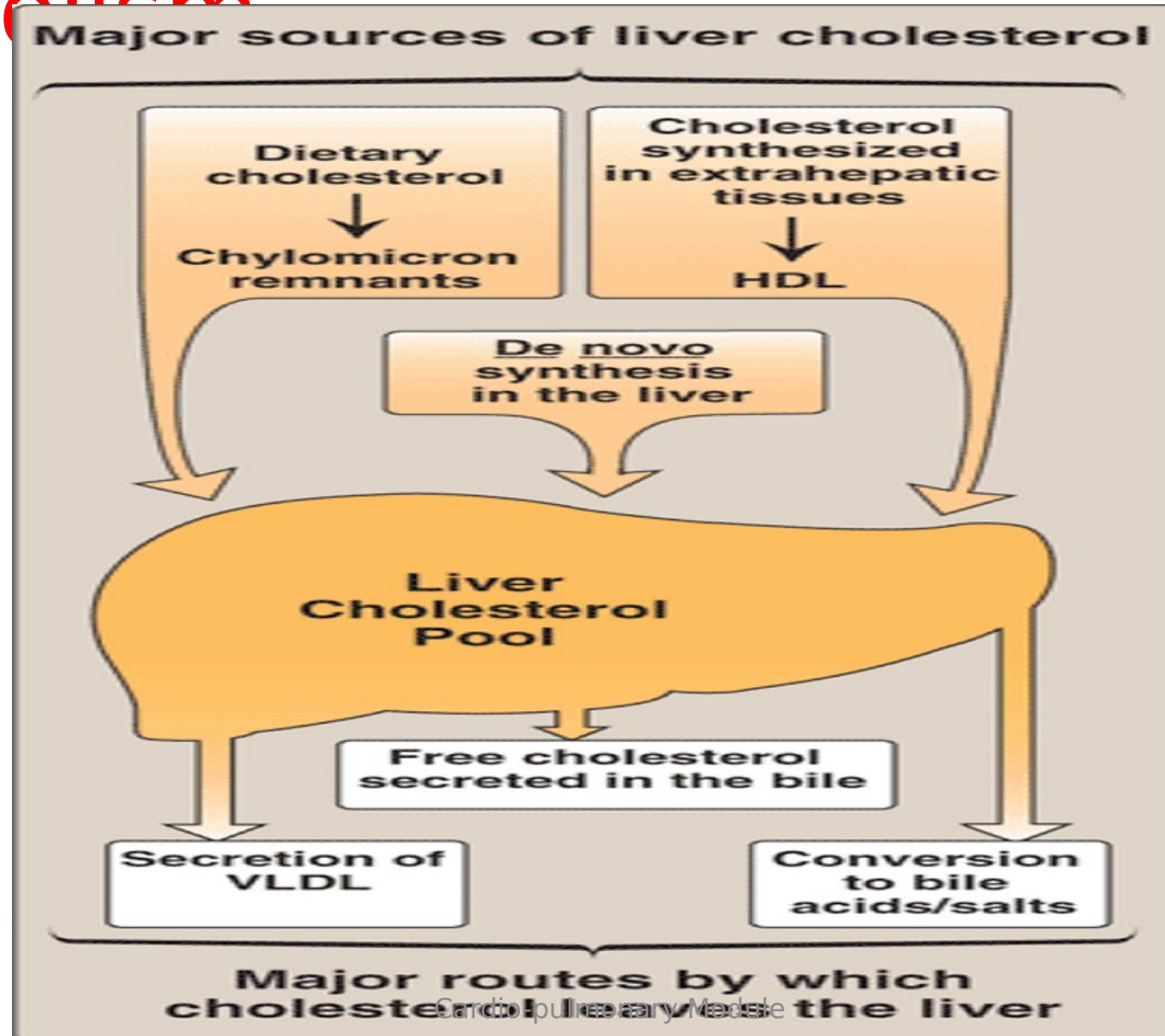
Which sentence accurately describes cholesterol?

- A. The final product of cholesterol catabolism is CO_2 and H_2O
- B. It is formed of a steroid nucleus with a 12 hydrocarbon side chain at C17
- ☒ C. It has no double bonds
- D. It is reduced in the intestine into coprostanol
- E. Cholesterol esters are more hydrophilic than free cholesterol

How is cholesterol transported in the body?

- 1- Transported as Lipoprotein particle**
- 2- Solubilized by phospholipids and bile salts in the bile.**

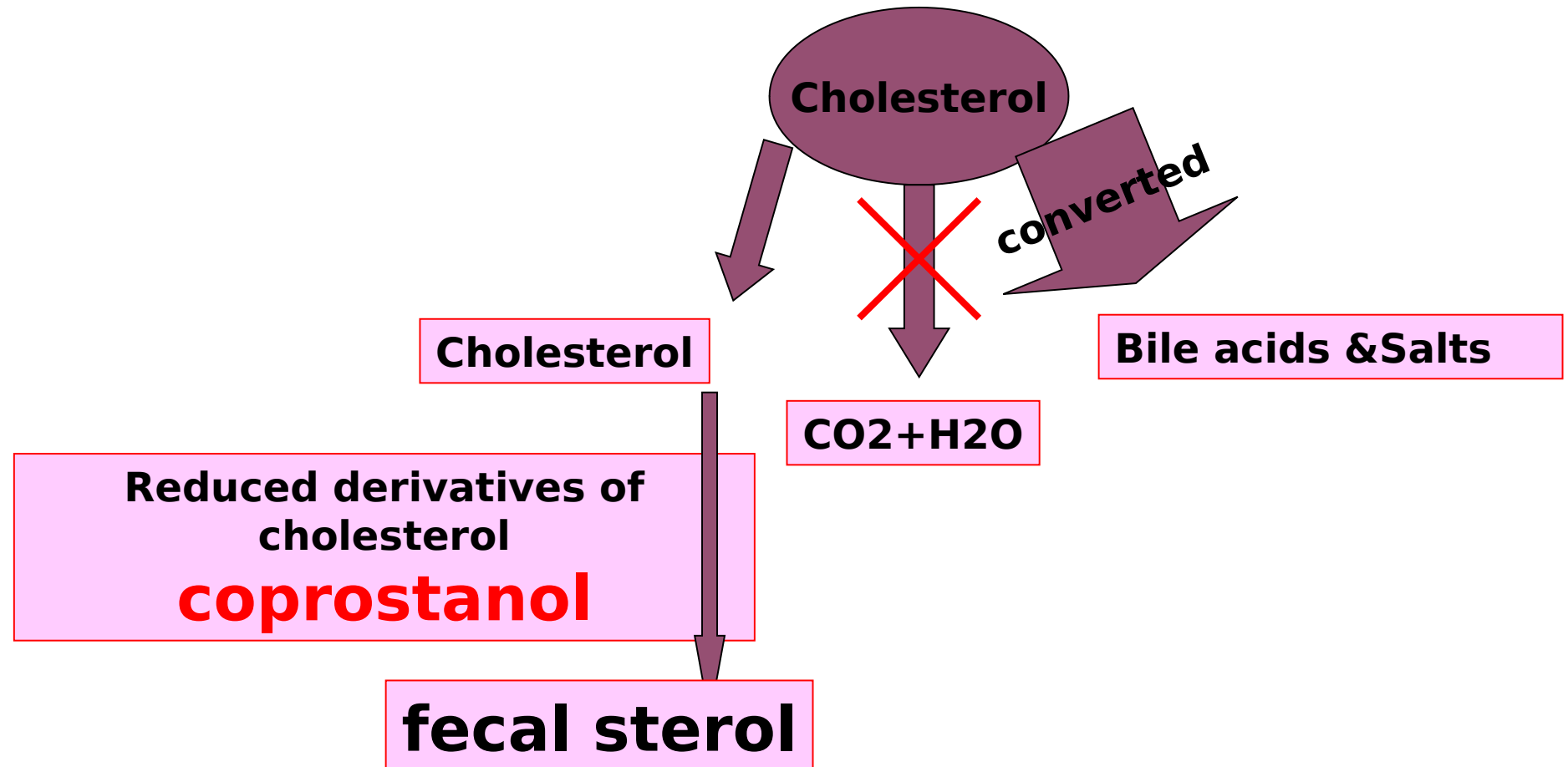
Describe role of liver in cholesterol metabolism



Which of the following statements about synthesis of cholesterol is WRONG?

- A. Cholesterol is synthesized in the liver only.**
- B. All its carbons are provided by acetyl CoA + NADPH+H⁺.**
- C. Synthesis occurs in the cytoplasm.**
- D. HMG CoA reductase is the rate-limiting enzyme catalyzing the key regulated step**

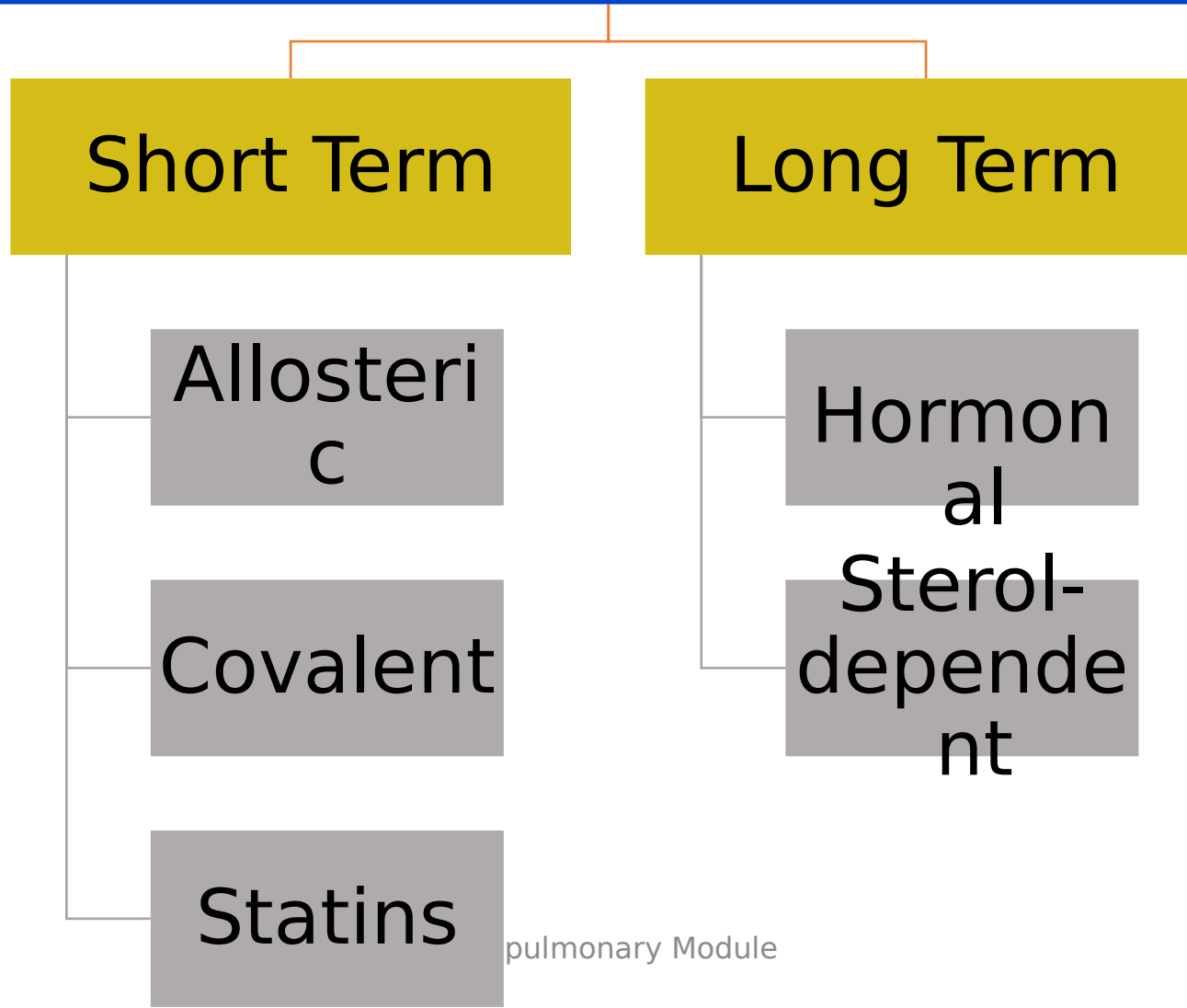
Describe the degradation and excretion of cholesterol



Discuss mechanisms of cholesterol synthesis regulation

**HMG-CoA reductase
(The committed/ The rate limiting step)**

Regulation of HMG- CoA reductase



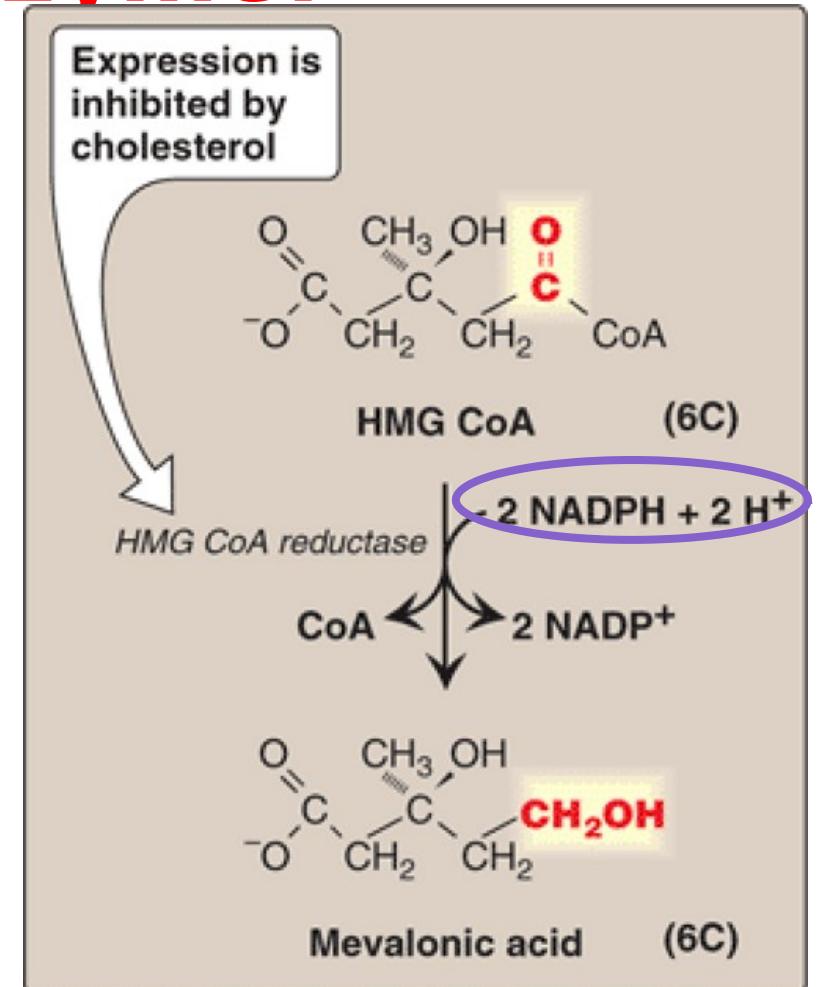
- Allosteric feedback inhibition by Mevalonate
 - Covalent modification (Phosphorylation by AMPK (↓ activity)- Dephosphorylation (↑ activity).
 - Inhibition by statins (competitive inhibition)
-
- Sterol dependent: High Cholesterol → ↓ Gene expression, ↑ proteolysis and vice versa.
 - Insulin, Thyroxin → ↑ Gene expression while Glucagon, glucocorticoids → ↓ Gene expression

Hypercholesterolemia is better to be treated by:

- a) NSAID
- b) Cortisol
- ☒ c) Statin drugs
- d) Glucagon hormone
- e) Bile salts

HMG-CoA reductase consumes.....as a coenzyme:

- a) 1 molecule of $\text{NADH} + \text{H}^+$
- b) 1 molecule $\text{NADPH} + \text{H}^+$
- c) 2 molecules of $\text{NADH} + \text{H}^+$**
- d) 2 molecules of $\text{NADHP} + \text{H}^+$
- e) 2 molecules of FAD



Key Points



- Cardiac biomarkers and isoenzymes in cases of MI.
- Covalent modification of pyruvate dehydrogenase enzyme.
- Steps of fatty acid synthesis and activation.
- Steps and regulation of TAG metabolism.
- Differences between fatty acid synthesis and oxidation.
- Carnitine: sources, functions, and its deficiency manifestations and ttt.
- Overview of lipoprotein metabolism and related defects.
- Cholesterol metabolism and its regulation.
- Hypercholesterolemia: manifestations and ttt.

References

- ▮ "Lippincott's Illustrated Reviews in Biochemistry" by P.C.Champe, R.A.Harvey and D.R.Ferrier
- ▮ "Harper's Biochemistry" by R.K.Murray, D.K.Granner, P.A. Mayes and V.W.Rodwell.
- ▮ Fundamentals of Clinical Chemistry (Tietz) Sixth
- ▮ "Textbook of Biochemistry with Clinical Correlations" by T.M.Devlin
- ▮ **www.namrata.co- *Biochemistry for medics***

*Thank
you*

